Preview of Award 1061695 - Final Project Report

Cover |
Accomplishments |
Products |
Participants/Organizations |
Impacts |
Changes/Problems

Cover

Federal Agency and Organization Element to Which Report is Submitted:	4900
Federal Grant or Other Identifying Number Assigned by Agency:	1061695
Project Title:	RUI: Colonization genetics of globally invasive marine bryozoa: Does adaptation prior or post-introduction determine spread?
PD/PI Name:	Sean F Craig, Principal Investigator Joshua mackie, Co-Principal Investigator
Recipient Organization:	Humboldt State University Foundation
Project/Grant Period:	03/15/2011 - 02/28/2015
Reporting Period:	03/01/2014 - 02/28/2015
Submitting Official (if other than PD\PI):	Sean F Craig Principal Investigator
Submission Date:	06/17/2015
Signature of Submitting Official (signature shall be submitted in accordance with agency specific instructions)	Sean F Craig

---

Accomplishments

*** What are the major goals of the project?**

The central goal of this project is to determine the extent to which genetic variation in colonial bryozoans in the genus *Watersipora* (a complex of cryptic species) predicts the success of invasions by these organisms in bays and harbors along the California coast. The study of a highly invasive colonial organism with different levels of genetic diversity within/across bays provides a means of determining whether ‘sorting’ of genotypes during the introduction process - or alternatively within local regions following introductions - results in rapid adaptation pre or post-introduction. We have

examined the following research questions:

(1) We examined phylogeographic patterns in the *Watersipora* complex, focusing on the Californian coastline using mitochondrial DNA and with microsatellite markers (after further development of microsatellite markers) as well.

(2) We used lab-based experiments at the Telonicher Marine Lab (Humboldt State University) to determine whether regional water temperature differences connect with phenotypic differences in these invasive bryozoans (in the Watersipora species complex) and help explain their pattern of invasion (in terms of the overall phylogeographic distribution).

(3) We used field experiments to investigate how copper tolerance (in antifouling paints) differs in different bays, influencing the pathways of invasions across multiple marine species in multiple harbors along the California coast. Copper, in the form of cuprous-oxide, is the active agent in antifouling paints currently used on all sizes of vessels. We expected many recently introduced fouling organisms, including *Watersipora* species, to be prime examples (being common and easy to study) of species with a high tolerance of dissolved copper. Centrally to this NSF funded project, we examined the importance of copper tolearnce to the spread of these invasive species within California.

(4) Last, but not least, a major goal was to help train future marine evolutionary ecologists, especially promising minority students, to play a lead role in biological research in the future. We have succeeded in this goal, by involving several very promising minority students through Humboldt State and San Jose State on to careers in science or to pursue Master's or PhD degrees at several academic institutions. One of our undergraduate students (Reginald Blackwell) even won a prestigious NSF-GFRP award (approx. $100,000) for study towards a PhD at Scripps Institute of Oceanography (starting fall 2014 in Dr. Ron Burton's lab-studying marine population genetics).

In this Final report-we record progress in completing experiments and writing up results for publication.

(We have included estimates for the percentage of completion for meaningful items below-these are shown in bold):

Year 1, 2011	June −Aug	Travel and collect samples along the entire California coast, from San Diego to Humboldt Bay, for genetic analysis. Beginning in September, 2011 samples were analyzed using COI & Microsatellite markers in the SJSU Conservation Genetics Lab. **(100% complete)**
	Sept-Dec	Continued genotyping and genetic analysis **(100% complete)**
Year 2, 2012	Jan-May	Continued genotyping and genetic analysis **(100%, one manuscript complete, with more to follow)**
	June-July	Collection of colonies along CA, establishment of cultures at HSU for common garden experiments. **(100% complete)**
	July −Aug	Commencement of experiments using cultured bryozoan stocks at the Telonicher Marine Lab of HSU. **(100% complete)**
	Sept-Oct	Finished common garden experiments at the Telonicher Marine Lab (TML), with 3 separate experiments each run for 6-7 months time **(100% complete)**
	Dec	Completion of a manuscript describing microsatellite variation. **(100% done: one manuscript describing marker isolation published). The thesis of Darren Wostenberg is complete (attached), and a results based manuscript is in progress.**
Year		

3, 2013	Jan-Aug	Completion of analysis of lab-based experiments at TML (Humboldt State Univ.). **(100% complete)**
2015	Jan-May	Completion of thesis (Korcheck) describing common-garden seawater temperature experiments is now complete, currently writing manuscript describing common garden experiments for publication. **(100%, Thesis complete (and attached), manuscipt in progress)**

*** What was accomplished under these goals (you must provide information for at least one of the 4 categories below)?**

Major Activities:

(1) We developed microsatellite markers for the common *Watersipora* species in California. These markers were generated through Illumina High Throughput paired-end sequencing, improving on an original set of eight microsatellite markers (provided by John Darling), which had limited amplification success of variability. These results have been pubilshed in a technical report (Mackie et al. 2014, Conservation Genetics Resources, **6**, 1053-1055) and are included in the thesis work of Darren Wostenberg (May, 2015) attached to this final report, and electronically selected amplifying primer pairs and microsatellite motifs (1000 each for two species) are supplied as a data resource.

(2) Data from temperature-related growth experiments at Humboldt State University's Telonicher Marine Laboratory (TML), started in summer of 2013, have been completed (2 years later-with 3 long-term experiments which ran 6-7 months each). In total, 361 colonies were monitored determining rates of growth and senescence, and mortality at a constant environmental temperature of 11dC or 18dC. The thesis of Kellan Korcheck, which focused on this work, has been completed (May, 2015) and is attached. A publication resulting from this work is in progress.

(3) The deployment of settlement panels dosed with peripheral paint strips, which release copper ions, creating a chemical gradient, was completed in the summer of 2014. This research allows us to asess intraspecific differences in copper tolerance *in Watersipora* populations as well as interspecific differences in copper tolerance across a spectrum of marine fouling organisms (data collected from 2012-2014). The survey sites have included recreational boat moorings, spanning highly copper contaminated areas (such as Americas Cup Marina and ShelterIslandHarbor in San Diego), and two areas of heavy commercial shipping, RichmondHarbor and Los AngelesHarbor. We have analyzed communities on 260 plates (in the field and via photographs), assessing copper tolerance in a range of phyla. These data are a valuable resource for assessing which species are copper-tolerant and possibly at higher risk of spread on painted hulls. A publication resulting from this work is in progress.

(4) The training of new students, both graduate and undergraduate, in a combination of molecular genetic techniques and marine science/biology in general on this NSF grant funded project has proven to be highly successful. Many (at least 5) of these students are now employed as scientists or teachers, or are continuing on in science by pursuing a graduate degree (Master's or PhD). In fact, with our help, one of our African American students (Reginald Blackwell) won a prestigious NSF-GRFP fellowship ($100,000) to pursue a PhD at Scripps Institute of Oceanography with Dr. Ron Burton, a leader in marine evolutionary genetics, which he started in the fall of 2014!

Specific Objectives:

Our specific objectives were as follows.

(1) We developed and used microsatellites and mitochondrial DNA (mtDNA) to the resolve taxomic status of cryptic species, as well as the invasion history and connectedness of populations of *Watersipora* species. Biological factors that affect these invasions include barriers to population establishment and maintenance due to temperature variations (Northern versus Southern California coast), and the use of copper as a fouling deterrant (which varies across harbors as well as across boat hulls painted with different copper paints).

Five Watersipora colonies were used separately to generate the genomic sequence of *Watersipora spp*. Microsatellites were developed using the data from one W. subtorquata and one W. n. sp. specimen. Sequences were analyzed using scripts described by Castoe et al. (2012). In each case, Illumina HT sequencing resulted in c. 10,000 potentially amplifiable microsatellite loci, illustrating the potential of such high throughput genetic technologies. The genome information has provided data sets for use in uiniversity classes on bioinformatics, and is being investigated to reveal genes of interest to further studies.

(2) Using experiments we directly determined whether genetic variation in *Watersipora* populations or across cryptic species predicts how life-history responds to differences in temperature. As such, the understanding of the genetic composition of *Waterspora* populations allows us to better understand heritable variation that may be adaptive and at least partially explain biological invasions. The work of Kellan Korcheck (see attached thesis) shows differences across bays as well as across genetic lineages in life-history schedules, as well as strong differences across Watersipora species in their tolerance to different temperature regimes. These results help to explain patterns of invasions by this bryozoan in space, and suggest evolutionary differences across bays.

(3) We utilized a method of comparing the copper tolerance of different organsims in marine fouling communities under natural situations, in which organisms settled and grew with (or without) dosed copper strips, testing broadly how significant the role of prior copper-tolerance (via genetic mutations) might be in the transport of invasive fouling organisms. This research has been completed-and results show copper tolerance in multiple species of marine invertebrates in bays all along the California coast. These results have been used to compare Watersipora subtorquata populations in California with those in Australia - allowing us to address whether the arrival of a genetically similar group of colonies, and subsequent spread over a segment of coastline, predicts copper tolerance over this invaded landscape.

The major objective of the project is to synthesize these findings to address whether mutation and natural selection prior to initial introductions, versus evolution in local areas of coastline (bays) subsequent to invasion, are important in determining the invasive potential of marine organisms.

Significant Results:

(1) Experimental use of molecular genetic markers

Microsatellite markers, along with a nuclear marker (to contrast with maternally inherited mtDNA), have now been used to genotype colonies collected at most of the sites of origin along the California coast. In areas where the major mtDNA clades defining *subtorquata* and *n. sp* populations occur separately, or coincidentally (e.g. Humboldt Bay, California), mtDNA-clade specific microsatellite genotypes have been observed. Thus the data gathered so far support the definition of *W. subtorquata* and *n*. sp as non-interbreeding species, fitting the Biological species concept. We carried out mitochondrial clade identifications of *Watersipora*

settling in the field (onto settlement panels dosed with copper strips) in different bays along the California coast. We found no settlement of *W. n. sp.,* with all observations of settlement being *Watersipora subtorquata* (clades A and B). This was true in the Eureka and Santa Cruz Marinas, where both *W. subtorquata* and *n. sp*. are common on docks, suggesting that the two species have different larval release timing, or different larval dispersal behaviors, at these sites.

A sample of 288 *Watersipora* colonies collected along the California coastline was genotyped at 6 microsatellite loci as well as the COI gene sequence. Bayesian clustering of genotypes into ideal populations consistently supported a match of microsatellite allelic variation to mitochondrial lineage. In total we observed 173 *subtorquata* and 115 *n. sp.* colonies in distinct population pools.

To assess spatial-genetic pattern, we subdivided the coast of California into a north coast region (Crescent City and Humboldt Bay), San Francisco Bay region (Tomales and SFB), a second central coast region (Santa Cruz and Monterey), and a South Coast region. Analysis of molecular variance (AMOVA) was used test for evidence of significant genetic differences within and among these regions in th introduced populations of *W. subtorquata* and n. sp . In *W. subtorquata there* was significant genetic segregation at the regional level *[F-statistic (F*RT*) of* -0.046 ($p < 0.001$)]. Also, subpopulations within each region *(F*SR = 0.058*)* tended to be differentiated from one another ($p = 0.001$). The new. sp. populations, sampled in northern California, SFB, and Monterey Bay, showed no significant segregation at the regional level [*F*RT = c. 0, the point of panmixia ($p = c. 0.999$)], but differentiation was found among subpopulations within regions (*F*SR = 0.192, $p < 0.001$). *Watersipora* n. sp. has not been found to occur in southern California, a limit to its distribution likely caused by warm water intolerance (see common garden experiments below).

The ability to infer species using mitochondrial clade identification lead us to develop the use of rapid mtDNA multiplex PCR typing (Laruson et al. 2012) to investigate distribution patterns of *subtorquata*/new. sp. in large numbers over finely measured environmental variable gradients.

Focusing our investigation in the San Francisco Bay coast region, we obtained temperature measurements, including seasonal average, minima and extemes, and salinities across a span of seven years (2008-2014). Environmental variables were then related to to *Watersipora* colony collections. Colonies were collected from floating boat moorings, in other words, a repeated experimental subtrate (negating some of the effects of other habitat variables).

In 2014, *W. subtorquata* was the *only Watersipora* species collected at marinas around SFB with relatively warmer (2-3˚C) waters during summer. The absence of new sp. from these same habitat patches (mostly floating pontoons), supports the hypothesis of warm water temperature intolerance for new sp. Partial Mantel tests comparing all variables also supports summer temperature as the strongest predictor of the occurrence of these species. Life history comparisons over different temperatures (described below) provided direct support for these observations based on spatial patterns of occurrence. *Watersipora subtorquata* and n. sp. appear to be a non-interbreeding pair of cryptic species which have a mosaic distribution predicted by sea water temperature.

(2) Specific findings in life-history experiments examining effects of temperature

The specific findings of experiments examining the growth, reproduction and survival of *W. suborquata* and *W. n. sp.* populations are summarized as follows.

(i) *W*. n. sp. colonies had significantly lower survival at the warmer temperature (18 degrees C) in one experiment, showing 100% mortality. *W*. n. sp. generally survived at the lower experimental temperature (11 degrees C); *whereas W. subtorquata* colonies survived at both low and high temperatures, but showed significantly greater survival at the higher temperature.

(ii a.) Colony growth at the warm temperature was higher *for W. subtorquata* than *W.* n. sp.

(ii b.) Colony area did not show a simple linear response to temperature: *W. subtorquata grown* at 18 degrees C showed a lobed growth form, with new zooids budded along an irregular growth margin, giving colonies a clover-like appearance. In *W. subtorquata* grown at the cooler temperature, 5-8 months of growth resulted in highly circular colonies with a regular perimeter of newly budded zooids. Hence colony shape and zooidal deployment differ in *W. subtorquata* across temperature environments.

(iii) Larval production was highly variable in these lab experiments. Although *W.* n. sp colonies produced relatively few brooded larvae in our experiments, *W. new sp.* generally produced none, even over the long span (7-8 months) of our experiments. The collection site of parental colonies of *W. subtorquata* was a significant factor affecting both the frequency of brooded larvae and the timing of first reproduction of these larvae-suggesting differential evolution across bays.

In summary, the observed mortality patterns and overall growth rate comparisons (observations i & iia) supported the temperature-dependent fitness hypothesis. Phenotypic differences were supported as the explaination for the geographic patterns of abundance of different Watersipora clades: the center of density of *W. subtorquata* (common in warmer areas of the west US coast) matched its higher performance in warm water in common-garden experiments in the lab, while *W.* n. sp is more common in cooler areas and showed higher peformance in colder water in the lab.

(3) The hypothesis that copper tolerance explains the spread of *Watersipora* species and other invasive fouling organisms

'Arrays' of panels which consisted of one non-dosed control plate along with plates with either a low or high dose of copper (a variation on Johnston and Webb 2000) were deployed in small vessel marinas in California from the middle of July, 2012, 2013, 2014 for periods of c. six weeks each. In summer 2013, the panel experiment effort was centered in marinas in San Diego California. In summer 2014, we obtained access (by collaboration with the California State Lands Commission Marine Invasive Species program and their Personnel) to shipping terminals in Richmond and Los Angeles, California.

Within these coastal zones we chose experimental areas of extremely high copper pollution and areas of lower copper pollution to test the hypothesis that local adaptation to copper pollution might affect the settlement and growth responses of fouling organisms. We observed similar copper tolerance of *W. subtorquata* colonies regardless of the background level of copper in the water. In contrast, some of the other introduced species, including *Hydroides elegans* (a tube worm) and some native species (such as Celleporaria brunnea, an encrusting bryozoan

which is comparatively intolerant of copper), showed signatures of higher copper tolerance in some copper pollution "hotspots", suggesting local adaptation.

Key outcomes or Other achievements:

In this reporting period we achieved a major aim of the proposal, using nuclear gene markers (microsatellites) to address the invasion ecology of *Watersipora* in detail.

The microsatellite marker development resolved an issue identified previously: the lack of easily amplifiable, variable genetic markers. We have begun generating data to test hypotheses regarding the role of environmental factors, including temperature and shipping traffic levels, in determining invasion patterns using collections of *Watersipora* colonies from throughout the US west coast. Generally, the multiple gene loci allow us to test dispersal pathways (commenced in Wostenberg's thesis, to be analytically extended in the publication), to make inferences about the numbers of founders in invasions using linkage equilibrium and Bayesian estimates of population sizes from phylogenetic information. A manuscript currently in progress describes this research.

The genetic data support the identity of two species of Watersipora which were also shown (in lab common garden experiments) to be quite distinct ecologically. The genetic signatures of *Watersipora subtorquata* and *n. sp.,* studied extensively in the field along the coast of California, suggest barriers to hybridization. Whether the relevant barrier is pre-fertilization incompatability of gametes, differences in the timing of release of gametes, or incompatibility due to differences in chromosomes (or some other biological pathway such as incompatability manifesting at or after sperm-egg fertilization) remains an open question.

According to microsatellite data for Watersipora *subtorquata* and n. sp. populations in California, there was similar regional-level and local population differentiation. Small-scale difference in genetic variation, which is high, fits with the idea that stochasticity (genetic drift) strongly influences the genetic variation of populations colonizing different bays.

In both bryozoan species, we identified signals consistent with the presence of clonal ‘patches’ – in other words sample sites were enriched in the occurrence of identical biparental genotypes, indicating local effects of asexual reproduction. The scale and life history patterns that result in asexual reproduction need further investigation, as the pattern may be consistent with propagative growth of colonies (ramet formation), or possibly spawning of clonally generated larvae (e.g. diploid parthenogenesis could not be ruled out).

As described above, information from the comparative life history experiments supports differing temperature-dependent fitness patterns and helps to explain the abundance of *W. subtorquata* and W. n. sp. in different areas along the California coast (and in particular, the absence of *W.* n. sp. in warmer waters).

Central to the goals of the grant, we successfully tested whether the distribution of W. *subtorquata* and n. sp at smaller, local spatial scales reflects water temperature influences or other patterns, such as salinity gradients-both in Humboldt Bay and in San Francisco Bay. A paper describing the pattern of these invasions within these two bays is in preparation.

Further (a goal in preparation, shown below) we will report on species-level patterns of copper tolerance measured using fouling panel experiments in the field during 2012-14. *W. subtorquata*, recently introduced to California, was found to be

relatively tolerant of copper, showing coverage that was not different on control and treated panels. Understanding the genetic adaptatations to copper in W.subtorquata may be served by investigation of intraspecific variation measurable among *W. subtorquata* populations. We have examined the potential for correlation with genetic lineage, or the copper pollution levels of the sites of occupation. Determining whether the level of copper tolerance ties directly to the potential for introduction could be further facilitated by examining the genetic lineages found on ship hulls, their genetic lineages, and the geographic distribution of these lineages, as well as through extension of fouling panel experiments used thus far to different coastlines in the future.

There was no evidence to support obvious patterns of local adaptation to copper occuring within California among the introduced *W. subtorquata* populations. Evidence of possible continental scale founder effects that may leave a persistent 'signature' of higher or lower copper tolerance is currently being addressed by measurement of copper tolerance in southern Australia. The copper tolerance of recently-introduced populations of *W. subtorquata* in California, however, did not appear as high as those measured in Australia, (Mackie unpubl. data-reported in the NSF proposal). Panels were deployed in South Australia (Northhaven Marina, Adelaide) and Victoria (Williamstown, near Melbourne) in March-April, 2015 to test the hypothesis that a continent-scale series of founder effects may be relevant to predicting copper tolerance. So far, *W. subtorquata* recruitment was high at both sites, and quantification of this data (images of settlement panels) is still in progress.

*** What opportunities for training and professional development has the project provided?**

This project provided multiple opportunities for undergraduate and graduate students at both institutions for training and professional development. There were a total of 12 results-based presentations given at scientific conferences by students involved in this project who worked with Drs. Craig and/or Mackie, many of which involved oral and/or poster presentations given by the students themselves (and not simply as co-authors).

Presentation of results at scientific meetings in some cases provided opportunities for students to gain experience in writing funding applications. Darren Wostenberg (SJSU) received a travel grant from the California State University Council on Ocean Affairs, Science and Technology to attend the Pacific Estuarine Research Society Conference in Apri, 2014 (in Newport, Oregon), where he presented results on the use of microsatellites (and recognized in the PERS Society Newsletter). Priyadarshini Iyer similarly (also assisted by COAST and NSF funding) presented on aspects of fouling panel studies at the Western Society of Naturalists Meeting, November 2013 (in Oxnard, California). NSF funding enabled four students along with Drs Craig and Mackie to attend a highly focused international conference - the 8th International Conference on Marine Bio-invasions (in Vancouver, Canada, August 2013). This conference involved three presentations from our group, with two being given by students themselves (1 graduate student = Kellan Korcheck and 1 undergraduate student = Pamela Ward), facilitating growth in oral communication skills on an international stage.

As an example of highly interactive periods of research involving student trainees, the summer of 2013, this grant provided summer fellowship experience for five undergraduate students at SJSU. Two of these students conducting summer fellowships are now in Biology programs at the University of Berkeley, while one is continuing in her Biology undergraduate program at the University of California at Santa Cruz.

Further, the project has provided the opportunity for engagement with expert professionals in Information Technology (Doan, DeMeo, Kam). These interactions in developing publications greatly assisted our skill set, and enhanced synergy between bioinformatics and ecology. Many of the experiences were used in courses involving a surveys of biology and bioinformatics tools (designed and taught as Bio-255E, Eco-Informatics, and Bio-180, Topics in Computational Biology and Bioinformatics) by Mackie at SJSU. The courses were able to provide practical exposure to the chemistries used in DNA sequencing, practicalities of computing (including an introduction to system setup and

coding principles, which was provided in part by Dr Steven Haddock of the Monterey Bay Aquarium Research Institute as a guest presenter in each course).

This experience has of course been incredibly rewarding and meaningful in professional development to the two co-PIs. Mackie presented a Guest Lectures on marine ecology and climate change, ('How marine organisms are responding to environmental change, and the role of shipping in introductions') as part of the SJSU GreenTalk, series, an essential Environmental Science component taken by 450 Engineering students at SJSU. Though a conference, we and others related research experiences to teaching experiences. Mackie was Chair of the Local Organizing Committee for the, 2nd Life Discovery – Doing Science Conference, held at San Jose State University (Oct 2-5, 2014). The conference involving participants, teachers and college instructors and other professionals from 22 US states, UK, and Canada) centered on the topic of national teaching reforms and active teaching methods. Within this event we held a workshop on Bioinformatics for teachers (http://www.esa.org/ldc/).

Hands-on experience in different aspects of this project assisted in the progression/development of undergraduate students to the next stages of their careers involving Science at both institutions (San Jose State University & Humboldt State University). Several of our students (N= 4) have gone on to other paid scientific positions (eg., Bangal, Martin, Ho, Chen) or to further study in Biology in graduate programs (N=4, including Reginald Blackwell, Danielle Perryman, Aki Laruson, and Parham Tabar). Notably, 3 of the students involved in this research project (N=12 total undergraduates) who went on to further graduate work were minority students, all of whom (100%) are now in graduate school pursuing Master's (n=2) or PhD degrees (n=1). Two of these students are African Americans, a group very poorly represented in marine science, who were recently accepted into graduate programs: (1) Danielle Perryman (SJSU), who was supported through the NSF-RISE program at SJSU, graduated and was accepted to Dr Jennifer Grindstaff's Laboratory at Oklahoma State University to conduct graduate research into endocrine systems and ecology in their Masters-PhD program, and (2) Reginald Blackwell (HSU), who was supported by an NSF-URM program as well as this NSF-RUI grant, graduated and was accepted into the PhD program to work in Dr. Ron Burton's Laboratory at Scripps Institute of Oceanography, contiuning study of marine molecular ecology. In fact, Regginald Blackwell landed a prestigious 3-year NSF pre-doctoral GFRP fellowship (approx $100,000) to pursue this degree!

All told, this project helped to spur on the completion of undergraduate degrees by eleven (11) of the twelve (12) undergraduate students involved (1 is still enrolled at UC Berkeley to complete his degree). In addition, of the 12 undergraduate students (total) involved in this NSF funded project, seven (7/12 = 58%) have gone on to graduate programs in the sciences.

This project has also lead to a great deal of productivity by our graduate students! A total of 14 graduate students have been supported, at least in part, by this NSF grant. Eleven (11) have completed Master's theses related to this research project. Three Master's students completed the graduate program at San Jose State University involving research under this NSF project: Priyadarshini Iyer, Niusha Taedi, Darren Wostenberg (whose population genetic work is central to this project, thesis attached). Kent Susick at SJSU is currently working on a Master's thesis examining the strength of boat traffic and copper pollution as variables explaining exotic fouling species abundance across habitats around California. Six completed theses at Humboldt State University related to this NSF grant: (1) Kellan Korcheck completed her Master's degree in May, 2015: her common-garden experiments examining Watersipora at the Telonicher Marine Lab (attached) are central to this project (paper in preparation), (2) Julie Kelly completed her Master's thesis in May, 2014 which, although focused on work on marine nudibranchs, benefitted from genetic work (COI sequences) done at SJSU (paper in preparation), (3) Nick Perkins, visiting grad student from the University of Tasmania (Australia) completed research examining how the complexity of *Watersipora* colonies relates to the community of epibiotic organsims held within them, (4) Emily Wilson completed her Master's thesis (attached) focusing on the facilitation of organisms in/on 3-dimensional *Watersipora* colonies in Humboldt Bay, California, (5) Josh Peterson completed a Master's thesis in mathematics (attached) in which he modeled the growth of *Watersipora* colonies in Kellan Korcheck's experiments (paper in preparation), and (6) Katie Houle completed a Master's thesis focusing on how sedimentation in Humboldt Bay effects fouling organsims (including *Watersipora*) of encrusting and upright growth forms (paper to follow). Finally, one additional grad student (Jason Lopiccolo) just began his graduate thesis work at HSU on *Watersipora* this Spring (2015) semester.

We are very proud of the accomplishments of all of our students!

*** How have the results been disseminated to communities of interest?**

The results from this 4 year project have been presented in journal papers (four published, several more in preparation) and through multiple (N=11) conference presentations: (1) A technical publication describing methods used to rapidly identify different mitochondrial phylogroups of *Watersipora* was published in Conservation Genetics Resources (2012), (2) A research paper in Scientific Reports (Nature) was published desscribing the latitudinal segregation of *Watersipora* invasions (2012), (3) A technical publication describing the PCR primers used in microsatellite analysis and the variability of these markers was published in Conservation Genetics Resources (2014), and (4) a fourth paper, related to the general theme of this research, on patterns of endemism in other byrozoans (Bugula) was also published (2014). In addition, several more papers are in preparation to be published in research journals describing (1) common garden experiments showing strong species-specific differences in *Watersipora* clades in temperature tolerance which reflect their invasion patterns (korcheck), (2) Mathematical models of the 2-dimensional growth of Watersipora colonies accurately predict the shape of colonies in warm water lab experiments (Peterson), (3)Marked differentiation among Watersipora populations in different bays and harbors at multiple microsatellite loci (Wostenberg), (4) Facilitation by Watersipora of multiple species inside/on Watersipora colonies in Humboldt Bay, California (Wilson), (5) The fractal dimension of Watersipora colonies accurately predicts the species richness of the fauna within them (Perkins), (6) Differentiation in spots on the dorsum of the nudibranch Daiulula sandiensis along with mating prefences and COI sequences reveal a second species (Kelly), (7) High sedimentation from winter storms in Humboldt bay differentially effect marine fouling organsims with encrusting versus upright growth forms (Houle).

The final (4th) paper related to this project which was published (2014) in conjunction with researchers in Brazil, Germany, and the US explored patterns of endemism in other bryozoans (in the *Bugula neritina* species complex), which are the source of important bioactive chemicals (bryostatins) currently being used to treat leukemia (as explained below).

Most of these results were presented at multiple conferences focused on marine biology and biological invasions, including meetings of the Western Society of Naturalists, the Pacific Estuarine Research Society, and the 8th International Conference on Marine Bio-Invasions. In addition parts of our research have been described to the public through GATE classes at local schools, training events for outreach with graduate students and local professionals, as well as other local venues (see below).

Products

Books

Book Chapters

Conference Papers and Presentations

Mackie, J., Martin, K., Craig, S. (2013). *Assessment of antifouling paint (copper) tolerance across common fouling organisms*. 8th International Conference on Marine BioInvasions. Vancouver, Canada. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Succow, Michelle S., Lamb, Catherine A., Mackie, Joshua A., Craig, Sean F. (2013). *COMPETITIVE INTERACTIONS BETWEEN CRYPTIC SPECIES – AN INVESTGATION OF THE WATERSIPORA SUBTORQUATA (BRYOZOA) COMPLEX*. Western Society of Naturalists. Oxnard, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Elder K. B., Craig, S.F. (2012). *Contrasting copper tolerance in two encrusting bryozoans: an invasive versus a native species*. Western Society of Naturalists. Seaside, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Priya Iyer & Joshua Mackie (2013). *Copper uptake rates of common invertebrates in marinas, an environment affected by boats and boat related introductions*. Western Society of Naturalists. Oxnard, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Korcheck, K.M., Kelly, J.A., Craig, S.F. (2012). *Crypsis of Diaulula sandiegensis on its sponge prey Haliclona permollis*. CSU COAST Faculty-student Research Poster Reception Featuring WRPI. Long Beach, CA. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Ward, Pamela K. & Craig, Sean F. (2013). *Defining environmental cues of the red alga Prionitis spp. that induce larval settlement of the invasive bryozoan Watersipora spp.*. Western Society of Naturalists. Oxnard, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Ward, Pamela K., Craig, Sean F. and Mackie, Joshua A. (2013). *Defining environmental cues of the red alga Prionitis that induce larval settlement of the invasive bryozoan Watersipora spp.*. 8th International Conference on Marine BioInvasions. Vancouver, Canada. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Blackwell, Reginald C., Craig, Sean F., and Mackie, Joshua A. (2013). *INVASIONS WITHIN HUMBOLDT BAY, CALIFORNIA BY CRYPTIC SPECIES OF BRYOZOANS (WATERSIPORA SPP.): SPATIAL AND TEMPORAL DOMINANCE BY THREE CLADES*. Western Society of Naturalists. Oxnard, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Wostenberg, Darren Mackie, Joshua (2014). *Investigation of population structure and distribution of the invasive bryozoan Watersipora species along the California coast using nuclear and mitochondrial DNA*. 37th Annual Pacific Esutarine Research (PERS) Meeting. Newport, Oregon. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Korcheck, Kellan, Craig, Sean F. and Mackie, Joshua A. (2013). *Population variation in temperature tolerance in a widely invasive bryozoan species complex (Watersipora spp.).*. 8th International Conference on Marine BioInvasions. Vancouver, Canada. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Korcheck K, Succow M, Guerrero A, Craig S, Mackie J. (2012). *Population variation in temperature tolerance in a widely invasive bryozoan species complex (Watersipora spp.). Research Poster*. Western Society of Naturalists. Seaside, California. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Inventions

Journals

Fehlauer-Ale, K.H., Mackie, J.A., Lim-Fong, G.E., Ezequiel, A., Pie, M.R., Waeschenbach, A. (2014). Cryptic species in the cosmopolitan Bugula neritina complex (Bryozoa, Cheilostomata).. *Zoologica Scripta*. 43 193. Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes ; DOI: 10.1111/zsc.12042

Joshua A. Mackie, John A. Darling & Jonathan B. Geller (2012). Ecology of cryptic invasions: latitudinal segregation among Watersipora (Bryozoa) species. *Scientific Reports*. 2 (871), 1. Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes ; DOI: 10.1038/srep00871

Kelly, J.A., Mackie, J.A., Craig, S.F. (2015). ASSORTATIVE MATING, GENETIC DIFFERENTIATION, AND DISTRIBUTION OF DISTINCT LINEAGES OF DIAULULA SANDIEGENSIS (Cooper, 1863). *Journal of Experimental Marine Biology and Ecology*. . Status = AWAITING_PUBLICATION; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes

Mackie, J. A., Wostenberg, D., Doan, M., Craig, S. F., Darling, J. A. (2014). High-Throughout Illumina sequencing and microsatellite design in Watersipora (Bryozoa), a complex of invasive species. *Conservation Genetic Resources*. 6 (4), http://link.springer. Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes ; DOI: 10.1007/s12686-014-0286-5

McCann, L. D,, Mackie, J. A., Ruiz, G. M. (2015). Mitochondrial DNA in the bryozoan Membranipora chesapeakensis (Banta, Perez and Santagata, 1995) — evidence of a recent introduction to both coasts of the United States. *Zootaxa*. . Status = SUBMITTED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes

Perkins, N., Craig, S.F., Macke, J.A. (2015). Measuring the effects of complexity and small-scale spatial variation on

facilitation by the ecosystem engineer Watersipora sp.. *will be submitted to ??*. . Status = AWAITING_PUBLICATION; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes

Áki J. Láruson, Sean F. Craig, Kirk J. Messer, Joshua A. Mackie (2012). Rapid and reliable inference of mitochondrial phylogroups among Watersipora species, an invasive group of ship-fouling species (Bryozoa, Cheilostomata). *Conservation Genetics Resources*. 4 (3), 617. Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes ; DOI: 10.1007/s12686-012-9606-9

Licenses

Other Products

Other Publications

Ho, A., Martin, K., Perryman, D., Wostenberg, D., Mackie, J.A., Bros-Seeman, S., and Craig, S.F. (2013). *Assessment of anti-fouling paint (copper) tolerance across common fouling organisms. Presentation (poster) at San Jose State University research symposium*. Poster presentation at San Jose State University Research Symposium. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Patents

Technologies or Techniques

Thesis/Dissertations

Websites

Supporting Files

Filename	Description	Uploaded By	Uploaded On
Wilson_Emily_Final_Version.pdf	Master's Thesis of Emily Wilson	Sean Craig	06/13/2015
peterson_joshua_m_sp2014-chopped.pdf	Master's Thesis of Joshua Peterson	Sean Craig	06/14/2015
KKorcheck_thesis_2015.pdf	Mater's Thesis of Kellan Korcheck	Sean Craig	06/14/2015
WostenbergThesisFinalSJSU2015.pdf	Master's Thesis of Darren Wostenberg	Sean Craig	06/17/2015

Participants/Organizations

What individuals have worked on the project?

Name	Most Senior Project Role	Nearest Person Month Worked
Craig, Sean	PD/PI	6
Mackie, Joshua	Co PD/PI	8
Bott, Nathan	Faculty	1

Parr, Leslee	Faculty	1
McMasters, Lynn	Other Professional	1
Nedelcheva, Raya	Other Professional	1
Scianni, Chris	Other Professional	1
Peters, Kristian	Staff Scientist (doctoral level)	1
Piola, Richard	Staff Scientist (doctoral level)	1
Bros-Seemann, Shannon	Statistician	1
DeMeo, Dustin	Graduate Student (research assistant)	1
Houle, Katie	Graduate Student (research assistant)	5
Iyer, Priyadarshini	Graduate Student (research assistant)	1
Kelly, Julie	Graduate Student (research assistant)	1
Korcheck, Kellan	Graduate Student (research assistant)	6
Laruson, Aki	Graduate Student (research assistant)	1
Lopiccolo, Jason	Graduate Student (research assistant)	5
Perkins, Nick	Graduate Student (research assistant)	1
Peterson, Misty	Graduate Student (research assistant)	1
Stiles, Sara	Graduate Student (research assistant)	1
Susick, Kent	Graduate Student (research assistant)	3
Taiedi, Niusha	Graduate Student (research assistant)	1
Wostenberg, Darren	Graduate Student (research assistant)	3
Chen, Jei-Ying	Non-Student Research Assistant	1
Ho, Ann	Non-Student Research Assistant	1
Martin, Kyle	Non-Student Research Assistant	1
Tabar, Parham	Non-Student Research Assistant	3

Blackwell, Reginald	Undergraduate Student	1
Chhina, Robinjeet	Undergraduate Student	2
Elder, Kathleen	Undergraduate Student	1
Roberts, Mary	Undergraduate Student	1
Sajadieh, Seena	Undergraduate Student	1
Succow, Michelle	Undergraduate Student	1
Ward, Pamela	Undergraduate Student	1
Doan, Michael	Consultant	1
Kam, Robert	Consultant	1
Perryman, Danielle	Research Experience for Undergraduates (REU) Participant	1

Full details of individuals who have worked on the project:

Sean F Craig
Email: sfc4@humboldt.edu
Most Senior Project Role: PD/PI
Nearest Person Month Worked: 6

Contribution to the Project: Dr. Craig has overseen all research on this project conducted at Humboldt State University (HSU), as well as coordinated with Dr. Mackie at San Jose State University and held joint lab meetings (at HSU) with members of both labs present. In addition-he has had several graduate students during this project whose research has touched upon and been supported by the topics of this grant: (1) kellan Korcheck-whose thesis centered on the common garden experiments central to this grant, (2) Emily Wilson, whose thesis focused on how the 3-dimensional habitat formed by Watersipora facilitates other species which live on/in it, (3) Nick Perkins, a visiting grad student from Tazmania whose thesis focused on the fractal dimension of Watersipora colonies, (4) Josh Peterson-who modeled the growth of Watersipora colonies in Kellan's experiments, and (5) Katie Houle-whose thesis focused on the effects of suspended sediments on Watersipora and other fouling marine organisms. Due to space limitations-only 3 of these these are attached to this final report.

Funding Support: This NSF grant

International Collaboration: No
International Travel: No

Joshua Adam Mackie
Email: joshua.mackie@sjsu.edu
Most Senior Project Role: Co PD/PI
Nearest Person Month Worked: 8

Contribution to the Project: As co-PI, organized experiments (lab and field), conducted research, and advised

students at San Jose State University. Master's students, undergraduate students, and paid research assistants were mentored in the Conservation Genetics Laboratory, SJSU.

Funding Support: This project (25%). Salary (Lecturer in Biological Sciences 75%).

International Collaboration: No
International Travel: No

Nathan Bott
Email: nathan.bott@rmit.edu.au
Most Senior Project Role: Faculty
Nearest Person Month Worked: 1

Contribution to the Project: Dr Nathan Bott coordinated the placement of settlement panels in southern Australia (March, 2015), and collections and genetic characterization of these specimens.

Funding Support: Faculty, Royal Melbourne Institute of Technology (RMIT), Melbourne.

International Collaboration: Yes, Australia
International Travel: No

Leslee A. Parr
Email: Leslee.parr@sjsu.edu
Most Senior Project Role: Faculty
Nearest Person Month Worked: 1

Contribution to the Project: Full Professor, NIH MARC and NSF RISE institutional lead. Shared lab space with J. Mackie. Co-advised Darren Wostenberg (thesis completed, spring, 2015). Assisted with grant administration.

Funding Support: SJSU Salary

International Collaboration: No
International Travel: No

Lynn McMasters
Email: lmcmasters@mlml.calstate.edu
Most Senior Project Role: Other Professional
Nearest Person Month Worked: 1

Contribution to the Project: Lynn McMasters is a graphic artist and affiliate of Moss Landing Marine Laboratories. Lynn has provided publication quality maps of bays and harbor installations studied in this project.

Funding Support: The current NSF award

International Collaboration: No
International Travel: No

Raya Nedelcheva
Email: Raya.Nedelcheva@slc.ca.gov
Most Senior Project Role: Other Professional
Nearest Person Month Worked: 1

Contribution to the Project: Research Officer, Marine Invasive Species Program. California State Lands Commission. Sacramento. Raya assisted Mackie in field experiments (August, 2014).

Funding Support: California State Lands Commission

International Collaboration: No
International Travel: No

Chris Scianni
Email: Chris.Scianni@slc.ca.gov
Most Senior Project Role: Other Professional
Nearest Person Month Worked: 1

Contribution to the Project: In 2014-15, Chris Scianni, Senior Environmental Scientist at Marine Invasive Species Program, California State Lands Commission, assisted in gaining permission to deploy settlement panels at industrial ports, and assisted in the fieldwork, and followup assessment of data and grant writing. The Marine Invasive Species Division of CSLC is taksed with monitoring movements of vessels and practices limiting the spread of exotic species via ballast water and hull fouling. The fouling panel methods used in this NSF funded project have provided the useful information for this agency monitoring effort - who share the same research concerns, including the need to accurately identify organisms from photographs, and the need to identify sources of introductions and pathways of spread.

Funding Support: California State Lands Commission.

International Collaboration: No
International Travel: No

Kristian Peters
Email: kristian.peters2@sa.gov.au
Most Senior Project Role: Staff Scientist (doctoral level)
Nearest Person Month Worked: 1

Contribution to the Project: Dr Peters is the Senior Scientific Officer/ Marine Research Coordinator for the Adelaide and Mount Lofty Ranges Region, Marine Research Division. Peters assisted by deploying panels in North Haven Marina, South Australia, and collecting photographic data (March-May, 2015). He is actively working on molecular assays and fouling species issues.

Funding Support: Department of Environment, Water and Natural Resources, South Australia.

International Collaboration: Yes, Australia
International Travel: No

Richard Piola
Email: Richard.Piola@dsto.defence.gov.au
Most Senior Project Role: Staff Scientist (doctoral level)
Nearest Person Month Worked: 1

Contribution to the Project: Dr Richard Piola deployed settlement panels and collected photographic data at the DSTO Research Pontoon (March-April, 2015). Piola's PhD and publication includes work on Watersipora, and bioinvasions, focusing on the role of copper tolerance.

Funding Support: Defence Science & Technology Organisation, Maritime Division, Melbourne VIC

International Collaboration: Yes, Australia
International Travel: No

Shannon Bros-Seemann
Email: shannon.bros@sjsu.edu
Most Senior Project Role: Statistician
Nearest Person Month Worked: 1

Contribution to the Project: Dr Bros-Seemann is an associate professor in Biological Sciences at SJSU, teaching courses on biostatistics and zoology. She provided valuable input in analysis for this project. Dr Bros is currently co-advising (with Mackie) a Master's student (Kent Susick) involved in continuing this research.

Funding Support: Other

International Collaboration: No
International Travel: No

Dustin DeMeo
Email: dustindemeo@hotmail.com
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Research graduate student, developed computation facility and approaches for genetic data analysis (SJSU Master's program). Dustin's input includes the development of scripts in Python. He is currently working with Mackie on an extension of the non-model genome characterization done in Watersipora, to develop a range of useful tools for applications in ecology and other more general areas of bioinformatics. A publication is expected on this work for submission in 2015.

Funding Support: This NSF-RUI grant has funded class project work as well as the development of further bioinformatics methods with Dustin DeMeo.

International Collaboration: No
International Travel: No

Katie Houle
Email: Katie.Houle@humboldt.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 5

Contribution to the Project: Katie Houle, a new grad student at Humboldt State University, joined the research team following the completion of her undergraduate degree at McGill University in Montreal, Canada in the fall of 2012. She has previous research experience on invasive species working with Dr. Ted Grosholz at UC Davis. Katie successfully defended her thesis proposal and advanced to candidacy in the spring (2013) semester. She conducted some experiments to examine whether the highly stratified spatial pattern of Watersipora clades found in Humboldt Bay (discovered by the research of undergraduate Reginald Blackwell, who also worked in the Craig lab) is due to local adaptation of these different clades to different water temperatures (and/or different salinity/turbidity/flow conditions) at different docks within our bay. Unfortunately-after several months of work, all the explanted colonies of Watersipora that Katie deployed under docks in Humboldt Bay died due to high sedimentation (apparently due to nearby construction work). Now, Katie has completely re-engineered her masters thesis and has conducted lab experiments at the Telonicher Marine Lab (at HSU) to simulate suspended sediment loads and their impacts on marine fouling communities. This work has now been completed and her results show some that some (but not all) taxa (in particular encrusting bryozoans-but not upright ones) are negatively impacted

by sedimentation, whereas upright growth forms (e.g. the bryozoan Bugula) arenot strongly effected by sedimentation. katie completed her master's thesis in spring 2015 semester-and is currently working on a manuscript from that thesis work for publication (summer 2015)

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Priyadarshini Iyer
Email: pri1484@gmail.com
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Priya Iyer received her undergraduate degree overseas (in India) and has worked on a graduate project at San Jose State University under the direction of Dr. Josh Mackie focusing on a search for genes which might control the ability of Watersipora to tolerate the high levels of copper found in the anti-fouling paint on ship's hulls (which is very likely a key adaptation that helps this group of species invade harbors all around the world). She has helped (along with Niusha Taidi) to assist in field collections of bryozoans as well as the running of microsatellite sizing (operating an ABI3100 sequencer at San Francisco State University). Priya Iyer was recently awarded a Council on Ocean Affairs, Science and Technology (COAST) award of $3,000 for her masters project: De-novo isolation and characterization of active copper-binding proteins in marine organisms. Priya presented this work (Western Society of Naturalists Meeting, Oxnard, California, poster). Priya completed a Master's (MA) Program in Biological Sciences (fall, 2013), through coursework and the research internship provided by this funding.

Funding Support: This NSF-RUI grant has funded research.

International Collaboration: No
International Travel: No

Julie Kelly
Email: jak40@humboldt.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Julie Kelly finished her master's thesis (defended April 2013) under the direction of Dr. Sean Craig at Humboldt State University. Julie was able to conduct a small population genetic study of cryptic sibling species populations of a shallow water nudibranch, Diaulula sandiegensis, with the assistance of Niusha Taidi and Josh Mackie at San Jose State in the Conservation Genetics lab. The information from this work is ready for publication in a paper (now fully written and close to submission) that combines information on life history variation, habitat variation, morphological variation (in spot number on the dorsum of this nudibranch) and genetic diversity to show that there are actually 2 different species of this nudibranch, with different spot patterns on their dorsum, which are found in different but overlapping geographic ranges along the Pacific Coast.

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Kellan Korcheck
Email: Kellan.Korcheck@humboldt.edu

Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 6

Contribution to the Project: Kellan was a graduate student working in Sean Craig's lab at Humboldt State University on this project whose thesis forms the central part of this grant project at HSU. Following a great deal of effort to collect live colonies of Watersipora of all 3 clades (A,B & "new species) from field sites up and down the entire California coast, she successfully got larval release from numerous sites, cultured hundreds of colonies, and ran several (3 long term-7 month each) experiments that included all 3 clades in both "Cold" and "Warm" water characteristic of Northern and Southern California waters, respectively. Results from this research are now complete-and Kellan finished her master's thesis in Spring 2015 semester at HSU. In addition, Drs. Craig & Mackie have met repeatedly with Kellan (via SKYPE and in person) and we have begun to draft a manuscript for submission to a journal for publication (as of June, 2015). We plan to finish that manuscript this (current) year (2015). Kellans results are terrific-and show that there is close to zero survival of the Northern clade ("new species") in warm water, and reduced reproductive output in cold water of southern clades (both Clades "A" and "B" of W. subtorquata). The responses of Watersipora to temperature-in terms of growth rate and survivorship-are very similar between clades "A" and "B", which makes sense given separate DNA analyses (showing close similarity between these clades). In addition-fascinating differences in the shape of colonies grown in warm versus cold water suggest strong differences in zooidal deployment (in space) and growth form (not just growth rate) which show extensive meandering, "searching" growth in more directive patterns (perhaps in search of better feeding micro-environments) in warmer water conditions in the laboratory.

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Aki Laruson
Email: akijl@hawaii.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Aki Laruson began on this project as an undergraduate at HSU, assisting with development of PCR techniques to identify the 3 clades of Watersipora studied in Humboldt Bay. He then went on to assist as a graduate student at the University of Hawaii, where he is currently pursuing a PhD degree under the direction of Dr. Dave Carlon.

Funding Support: this NSF award

International Collaboration: No
International Travel: No

Jason Lopiccolo
Email: Jal791@humboldt.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 5

Contribution to the Project: Jason has begun as a grad student at HSU in the lab of Sean Craig (PI) in Fall 2014, working on larval traits of Watersipora. Jason constructed his master's thesis proposal in the 2014-2015 AY, and this summer will collect colonies of Watersipora along the California coast to investigate their differences in larval traits (e.g. size, swimming ability) and how these traits effect adult growth characteristics (and other adult fitness aspects-including reproductive effort)

Funding Support: this NSF grant, along with CA SeaGrant support for ongoing Marine Protected Area work with

Dr. Sean Craig

International Collaboration: No
International Travel: No

Nick Perkins
Email: nperkins@postoffice.utas.edu.au
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Nick Perkins is continuing work on preparing/editing a manuscript for publication on the work he did while at Humboldt State University (HSU) pursuing a "masters" degree. The graduate exeprience allowed Nick to transition into the Biology PhD program at the University of Tasmania, Hobart. (Students take Honous or Master's preparatory degrees at most Austtralian universities, prior to PhD. This work resulting-during his exchange here in the US in the Fall 2011 semester (at HSU)-in his master's thesis through the University of Tasmania, where Nick is currently a PhD student. He is examining the ecological conditions including climate change, allowing the spread of the sea urchin Centrostephanus rodgersii southward in Australia, and ecosystem level effects.

Funding Support: Currently supported in Australia (Tasmania) as a PhD student there. (Nick is an Australian and traveled to the USA to work HERE as an international student)

International Collaboration: Yes, Australia
International Travel: No

Misty R. Peterson
Email: misty.peterson@sjsu.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Research graduate student at San Jose State University who assisted in genetic data analysis, and used ideas from that experience to develop a separate Master's project at SJSU (in collaboration with the University of California at Santa Cruz). At SJSU, her experience in Next Generation Sequencing as well as involvement in a project on the de novo sequencing of an invasive marine organism (Watersipora) is listed as an important experience in her online Bio as a PhD Candidate in Dr. Alessia Buscaino's Fungal Research group, University of Kent. "Her study at the University of Kent is under joint supervision of Dr. Alessia Buscaino and Dr. David Brown through a University of Kent 50th Anniversary Scholarship... takes a multi-disciplinary approach, integrating structure and functional studies of proteins that play a role in the morphological changes involved in the virulence of Candida albicans."

Funding Support: This NSF-RUI grant has funded class project work.

International Collaboration: No
International Travel: No

Sara Stiles
Email: sstiles6@gmail.com
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Sara Stiles was a brand new graduate student accepted for admission into the master's degree program at Humboldt State University for fall 2013, having completed her bachelor's degree in

Marine Biology at CSU-Long Beach (where she was a president's scholar, national merit scholar, and a Brotman science scholar) with a 3.9 GPA. She began to learn genetic techniques in the conservation genetics lab at San Jose State University under the direction of Dr. Josh Mackie in the summer (June-2013) and officially started in the grad program at HSU in fall (september) 2013. Following successful completion of her thesis proposal, along with courses including an advanced invertebrate zoology class (taught PI Sean Craig) which delved into bryozoan ecology extensively (particularly the patterns of life history variation they exhibit-the main topic of her thesis), Sarah decided to leave the graduate program at HSU in spring 2014. Although several faculty commented on what a good student she was, she felt the program was "stressful" and decided to rethink her direction. She is currently training as a (highly promising) high school Biology teacher.

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Kent Susick
Email: ksusick1@msn.com
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 3

Contribution to the Project: Kent Susick commenced a Master's program at HSU in fall 2012, and transferred to the SJSU Master's program, beginning 2014. Contributions to the project include assistng in settlement panel experiments, population genetic assays, and literature searches. Kent's Master's project extends from this award. Kent is using settlement panel surveys to address the questions about the extent of invasions of exotic species, comparing fouling communities close to boat anchorages and further away. Co-advised by Drs Bros-Seemann and Mackie. In summer 2015 Kent has been able to obtain a competitive fellowship, working with Dr Steve Lonhart (NOAA, Monterey Bay SIMON program). Expected Master's graduation: spring or summer 2016.

Funding Support: This project, Departmental Research award at SJSU, additional funding though Mackie.

International Collaboration: No
International Travel: No

Niusha Taiedi
Email: taidi_niusha@yahoo.com
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 1

Contribution to the Project: Niusha worked to help Julie Kelly sequence the COI region of the genome of the nudibranch Diaulula sandiegensis, which turns out to be a complex of two (possibly more) species. Niusha also assisted with development of microsatellites and fieldwork. sts Meeting, Oxnard, California, poster). Niusha completed a Master's (MA) Program in Biological Sciences (fall, 2012), through coursework and a teaching internship experience. She is currently working at Rainin (the Pipette company) in San Francisco.

Funding Support: This NSF-RUI grant award.

International Collaboration: No
International Travel: No

Darren Wostenberg
Email: dwostenb@hotmail.com
Most Senior Project Role: Graduate Student (research assistant)

Nearest Person Month Worked: 3

Contribution to the Project: Darren Wostenberg commenced the SJSU graduate program in fall 2012. He developed a thesis project based on the application of the microsatellite markers in the Watersipora system, with the goal of testing for hybridization occurring among ancestors of invading populations from different geographic locales, and to use the multi-locus microsatellite data to extrapolate information about the effective population size and spread of introduced founding populations. Darren completed this thesis in spring 2015 semester (accepted, Biological Sciences, SJSU). Darren has a Bachelor of Science degree in Fishery Biology and Wildlife Biology from Colorado State University, Fort Collins, CO (spring 2003), and experience as a research technician in terrestrial and aquatic systems. He is currently working for the US Department of Agriculture at Fort Collins, Colorado Animal and Plant Health Inspection Service. As a Biological Science Technician, Darren is working on rabies research, incuding employing molecular genetic techniques.

Funding Support: This NSF-RUI grant.

International Collaboration: No
International Travel: No

Jei-Ying Chen
Email: CChen@calacademy.org
Most Senior Project Role: Non-Student Research Assistant
Nearest Person Month Worked: 1

Contribution to the Project: Jei-Ying Chen is a graduate of San Fransisco State University who obtained her master's degree there. She assisted with molecular biology lab work at SJSU and assisted in fieldwork before taking a full-time position in Collections related Research at the California Academy of Sciences.

Funding Support: This NSF-RUI grant

International Collaboration: No
International Travel: No

Ann Ho
Email: ann.tq.ho@gmail.com
Most Senior Project Role: Non-Student Research Assistant
Nearest Person Month Worked: 1

Contribution to the Project: Ann Ho commenced work as an undergraduate student in the conservation genetics lab at San Jose State University under the direction of Dr. Josh Mackie. She helped to develop microsatellite markers for discrimination among different individuals (colonies) of Watersipora spp as well as with fieldwork. She was employed as a research assistant for 1.5 months in summer 2013. Ann is now a Clinical Laboratory Associate at Private Biotechnology Company, located in San Francisco.

Funding Support: This NSF-RUI grant

International Collaboration: No
International Travel: No

Kyle Martin
Email: kylmartin@yahoo.com
Most Senior Project Role: Non-Student Research Assistant
Nearest Person Month Worked: 1

Contribution to the Project: Kyle Martin started out as an undergraduate research assistant in the Conservation genetics lab at San Jose State University (SJSU) under Dr. Josh Mackie, and has since graduated (fall 2012) with his bachelor's degree from SJSU--but has continued on as a research-assistant who has worked hard to analyze the data for settlement panels with (and without) copper paint deployed at 5 different field sites (bays) along the coast of California. Kyle recently presented this work in poster form at a research forum at SJSU (in 2012). At the end of 2012, Kyle took up an equipment preparation technician position at Genentech, San Francisco.

Funding Support: This NSF-RUI award

International Collaboration: No
International Travel: No

Parham Tabar
Email: parhamcarlos@yahoo.com
Most Senior Project Role: Non-Student Research Assistant
Nearest Person Month Worked: 3

Contribution to the Project: In the final 1.5 years Paham, a graduate of SJSU (having taken classes with Mackie), volunteered in the Conservation Genetics Laboratory (1-2 days/week), conducting a wide range of research activities - genetic work and fieldwork. Parham has commenced the San Francisco State University M.S. in Oral Health Sciences Program, involving research with the option to convert to a pre-dental stream (commenced summer semester, 2015).

Funding Support: None.

International Collaboration: No
International Travel: No

Reginald Blackwell
Email: reggieb9@gmail.com
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Reginald (Reggie) Blackwell is an African American student who worked on this project for several years (2012-2014) and who has was awarded a prestigious NSF-REU internship followed by an NSF-GRFP scholarship (approx $100,000) to pursue a PhD at Scripps Institute of Oceanography in La Jolla, CA, working with Dr. Ron Burton on ecological genetics. Reggie started work as a PhD student in fall 2014 and is continuing on at Scrips now. while at HSU. Reggie was supported off a NSF-URM grant (to Prof. Bruce O'Gara and Matt Johnson) while an undergraduate at HSU, and chose to work in the lab of Prof. Craig (at HSU) to do his undergrad research. Reggie has been heavily involved in experiments examining the preference of Watersipora spp. larvae for different algal substrates, and presented a poster on this work with another undergraduate collaborator (Pamela Ward, also an NSF-URM fellow) at the SACNAS meeting in early fall, 2011. This meeting of the Society for the Advancement of Chicanos and Native Americans in Science was held in San Jose, CA from October 27-30th, 2011. Both Pamela and Reggie won an award ('Best Poster in Marine Science') for their presentation at this meeting. Reggie then went on to lead the work in our common genetic facility to investigate and determine the different clade types of Watersipora present within Humboldt Bay, and this work was presented at the 42nd Annual Benthic Ecology Meeting in Savannah, GA in spring 2013. This work will be combined with a model of hydrodynamic flow model (already constructed) to describe the highly stable patterns of abundance of all 3 clades of Watersipora around Humboldt bay and the flow patterns (modeled by a local hydrographer) which explain why these different clades are generally not intermixed. This paper is in progress.

Funding Support: NSF-URM grant (to Dr. Bruce O'Gara) as well as this NSF-RUI grant

International Collaboration: No
International Travel: No

Robinjeet Chhina
Email: robinchhina@outlook.com
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 2

Contribution to the Project: Through summer 2014 (3 months), Robinjeet assisted in labwork (microscopy, PCR, settlement panels constuction) and fieldwork. Robinjeet had taken an Associates Degree at DeAnza College, San Jose. He visited the SJSU campus during a student poster day, and was motivated to join our group of students as an intern the following month. Robinjeet is currently studying Biology (Pre-Med) with a Major in MicroBiology at UC San Diego.

Funding Support: This NSF-REU project.

International Collaboration: No
International Travel: No

Kathleen Elder
Email: kbe3@humboldt.edu
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Katie Elder was an undergraduate who worked on this project at HSU, presenting a poster of her work on Watersipora growth in the presence and absence of copper anti-fouling paint. After completing her undergraduate degree at HSU, she continued on the project as a technician for 1 summer 2012). She is currently working in Florida at the Mote Aquarium in the coral reef ecosystem research lab.

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Mary Roberts
Email: mmr31@humboldt.edu
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Mary assisted with the analysis of photos of colonies of Watersipora gathered by Kellan Korcheck (grad student at HSU) to transform photos into data on growth (area of colonies alive, area dead, number of brooded embryos, etc) in 2013 as part of the master's thesis project of kellan Korcheck. She has since graduated from HSU and left the project.

Funding Support: this NSF-RUI grant

International Collaboration: No
International Travel: No

Seena Sajadieh
Email: seenasajadieh@gmail.com

Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Seena, a first year student at Foothill Community College, San Jose, contributed time assisting with fouling panel construction, field work, and literature searching. Having completed introductory subjects in this time, Seena has now gone on to pursue a Bachelor's of Science (Psychology) degree at the University of Berkeley, commencing in fall, 2014.

Funding Support: This NSF award.

International Collaboration: No
International Travel: No

Michelle Succow
Email: msuccow08@gmail.com
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Michelle Succow began in the Craig lab at Humboldt State University in Fall, 2012 semester-helping with feeding and care of the Bryozoans we are culturing at our Telonicher Marine Lab(TML). She has set up up experiments to test for competition between different clades of Watersipora collected from different sites (for colonies of the same clade) in the laboratory at TML, and reported the results of this research (in a poster) at the Western Society of Naturalists mtg. She has now become a graduate student in Dr. Craig's lab, has completed her first year of graduate school (AY 2014-2015) and is currently working on her master's thesis researching another topic (connections between mole crab abundance and red surf perch along the sandy shores of Humboldt county) as part of an ongoing grant funded project working on the sandy beaches of nearby Marine Protected Areas.

Funding Support: this NSF-RUI grant (in past), and currently on an MPA grant for Sandy Beach Ecology (headed by Dr. Karina Nielsen, director of the Tiburon marine lab at San Francisco State University).

International Collaboration: No
International Travel: No

Pamela Ward
Email: pkw8@humboldt.edu
Most Senior Project Role: Undergraduate Student
Nearest Person Month Worked: 1

Contribution to the Project: Pamela has worked to investigate the preferences of Watersipora larvae for different algal species found growing along the docks in Humboldt Bay--having found them primarily settled on one alga (Prionitis spp.) which is common there. Her lab experiments have confirmed that this alga is indeed the primary preferred settlement site when given a choice between 4 algal species-and she is now testing to see if the reason for this preference is biological cues found on the surface of Prionitis. Pamela won 1st place in marine sciences for a poster she presented on this work in the fall (2012) meeting of SACNAS (Society for the Advancement of Chicanos and Native Americans in Science). She has also successfully graduated from HSU with her bachelor's degree (spring 2014) and has written a manuscript which is in preparation for publication this summer (2015).

Funding Support: both NSF-URM grant (to Dr. Bruce O'Gara at HSU) as well as this NSF-RUI grant

International Collaboration: No
International Travel: No

Michael Doan
Email: mythdo@hotmail.com
Most Senior Project Role: Consultant
Nearest Person Month Worked: 1

Contribution to the Project: Michael Doan is now a senior software engineer at ThermoFisher, South San Francisco (formerly Life Technologies Inc). Michael assisted in isolation of microsatellite loci from sequence data files, and coauthored a paper from this project.

Funding Support: None

International Collaboration: No
International Travel: No

Robert Kam
Email: rkam2001@hotmail.com
Most Senior Project Role: Consultant
Nearest Person Month Worked: 1

Contribution to the Project: Robert Kam, and Defense and Space Engineer, appoached Mackie about gaining epxerience in Bioinformatics. Through the final period, Kam assisted in running genome assemblies and other algorithms in large genomic data sets, using the Amazon web server. The contribution is expected to add to an initial characterization of the Watersipora genome.

Funding Support: This project.

International Collaboration: No
International Travel: No

Danielle Perryman
Email: Danielleclarisp@aol.com
Most Senior Project Role: Research Experience for Undergraduates (REU) Participant
Nearest Person Month Worked: 1

Contribution to the Project: Danielle Perryman, a Senior African American student at San Jose State University, has helped with DNA extractions and PCRs for the project in the Conservation Genetics Lab under the direction of Dr. Josh Mackie. She also assisted in the work to present a poster at the SJSU student poster session on variation in copper tolerance among bays in California (and she is co-author on poster presentations with other students). Danielle graduated from SJSU in fall 2014, and was accepted as a Master's student to Dr J. Grindstaff's Laboratory, Oklahoma State University, to conduct graduate research into endocrine systems and ecology of songbirds, within the OkSU Masters-PhD program.

Funding Support: This NSF-RUI grant, NSF-REU grant to SJSU.

International Collaboration: No
International Travel: No
Year of schooling completed: Other
Home Institution: San Jose State
Government fiscal year(s) was this REU participant supported: 2013, 2012

What other organizations have been involved as partners?

Name	Type of Partner Organization	Location
California State Land Commission, Marine Invasive Species Pr	State or Local Government	100 Howe Avenue. Suite 100 South, Sacramento CA 95825-8202
McKinleyville Middle School	School or School Systems	McKinleyville, California
Oregon Institute of Marine Biology	Academic Institution	Charleston, Oregon

Full details of organizations that have been involved as partners:

California State Land Commission, Marine Invasive Species Pr

Organization Type: State or Local Government
Organization Location: 100 Howe Avenue. Suite 100 South, Sacramento CA 95825-8202

Partner's Contribution to the Project:
In-Kind Support
Facilities
Collaborative Research
Personnel Exchanges

More Detail on Partner and Contribution: The California Marine Invasive Species Program (MISP) is charged with preventing or minimizing the introduction of nonindigenous species to California Waters from vessels over 300 gross registered tons, capable of carrying ballast water. In discussions with Nicole Dobroski, Environmental Program Manager, and Chris Scianni, Staff Environmental Scientist, MISP, we have discussed the potential to use their assistance in deploying settlement panels in shipping terminals in California in 2014, and the collection of specimens from dry-docked vessels for research. The exchange of information and collaboration is expected to widen the opportunity for experiments to be done to understand the impact of large-vessel fouling on greater coastline ecology.

McKinleyville Middle School

Organization Type: School or School Systems
Organization Location: McKinleyville, California

Partner's Contribution to the Project:
In-Kind Support
Facilities
Personnel Exchanges

More Detail on Partner and Contribution: Dr. Sean F. Craig presented a GATE course (3 sessions of 5 hours each = 15 contact hours total) for Gifted And Talented Education students in 4-8th grades (25 students total). This group learned from Dr. Craig (and live specimens he brought in to the McKinleyville Middle School) at their learning site as well as (for 1 day) at the Telonicher Marine Lab in Trinidad, California-home of this research project (this is the marine lab which is a part of Humboldt State University, where Watersipora colonies were raised).

Oregon Institute of Marine Biology

Organization Type: Academic Institution
Organization Location: Charleston, Oregon

Partner's Contribution to the Project:
Financial support
In-Kind Support
Collaborative Research

More Detail on Partner and Contribution: Dr. Jan Hodder at Oregon Institute of Marine Biology helped to arrange training in COSIA course development and supported the 1-week course offered at HSU for grad students/undergrads/local professionals in marine science outreach.

What other collaborators or contacts have been involved?
Nothing to report

Impacts

What is the impact on the development of the principal discipline(s) of the project?

The results of this research have wide-ranging implications for the study of biological invasions, particularly in the sea. Our results suggest that identification of cryptic species is vital for determining the pattern of invasions both within Bays and Harbors along the West Coast of the US, as well as across continents (e.g. along the Australian coast also). In addition-our results show strong effects of genetic background/lineage on invasion patterns in both space and time. The pattern of Watersipora invasions along the California coast clearly reflects sharp differences in the temperature tolerance of the two species examined (*W. subtorquata* and *W. n* sp.) which are impossible to distinguish without molecular-genetic techniques. These strong differences suggest that future changes in sea surface temperatures due to climate change will shift the pattern of invasions along our coasts. In addition, the evidence for genetic variation in microsatellite loci among/across bays in California suggests separate evolution of populations in different estuaries post-invasion, and life-history differences discovered in common garden lab experiments across sites of parental origin further support this phenomenon.

The evolution of copper tolerance in *Watersipora subtorquata* appears to have been a driver in its invasion success. But the evolutionary landcape of copper tolerance is not well understood. It is important to realize that despite much sampling the location of native (non-introduced) populations of *W. subtorquata* and n. sp. has not been narrowed down sufficiently to allow comparative tests of traits over native and introduced areas. Through the use of settlement panels we replicated measurment of copper tolerance in a standardized way across space, generating data for multiple marine fouling species. The copper tolerance phenotype was found to be generally related to invasion success, as tolerance was generally higher in the exotic fouling species than indigenous species. In some organisms, we found populations are responding locally to waterborne dissolved copper, with copper 'hotspot' areas being coincident with increased tolerance. But this pattern of localized difference in the overall variance of response was not observed in *W. subtorquata*. General tolerance of copper was seen in *W. subtorquata* throughout California. Considering the shoreline of Australia as an initial contrast, we are currently testing an alternative hypothesis that continental scale founder effects can be related to both population genetic marker signature of ancestry pattern and overall levels of copper.

The fact that many introduced fouling organisms are relatively tolerant of copper suggests that current man-made toxic chemicals, including copper in anti-fouling paints, may not be a viable long-term strategy to keep ship hulls clear of marine fouling organisms. Further development of new anti-fouling paints, along with additional study to determine whether tolerance to one anti-fouling paint comes at the expense of adaptation to another anti-fouling compound, is needed. Multidisciplinary pathways are likely to increase understanding of 'fouling' problems. Meaningfully, identifying the sources of variance in copper tolerance phenotype within species, as we are doing in *W. subtorquata*, holds

promise for identifying variations in genes that cause tolerance.

Ship traffic (causing propagule pressure) and the dissolved level of copper are meaningful variables for dissecting patterns of exotic species spread - and in guiding practices used for the maintenance of coastal organism diversity, which require study of boat traffic to remote areas. As a direct experimental impact, the results of this study have lead to a collaboration with the California State Lands Commission to study invasion pattern in Santa Catalina Island, California, a remote area, receiving nevertheless complex patterns of vessel traffic.

Finally, there is a general interest in the techniques developed in this study, including the use of gene sequencing to monitor the appearance of biological invaders using genetic methods, which is particularly important because of the prevalence of cryptic species in marine systems. One project, funded by the State of Victoria Government in Australia was influenced by the methods (settlement panels surveys) and genetic data obtained in this study. This project is entiteld: *Marine pest monitoring of the Gippsland Lakes* [Victoria, Australia, funding of $219,000 AU to Nathan Bott, RMIT University, Bundoora, VIC, Alastair Hirst (Deakin University, Waurn Ponds, VIC), Anthony Chariton (CSIRO Land & Water, Lucas Heights, NSW) & Joshua Mackie (San Jose State University, USA]. This project aims to explore the question of how genetic probe-based monitoring technologies, alongside traditional monitoring, can be used in detecting the spread of aquatic invaders.

What is the impact on other disciplines?

Ideas from invasion biology may be linked to research goals in active chemical compound research. To delve into this area, we have written an exploratory grant proposal (PI, Mackie) with two researchers in chemistry: Dr R. Okuda (SJSU) and Dr T. Amagata (San Francisco State), entitled "*Pharmacological and evolutionary perspectives on bioactive compounds in marine invertebrates - a focus on invasive species".* This CSU COAST (Council on Oceans Affairs, Science & Technology) development grant was funded. Work being conducted on the theme of toxicity has included a comparison of the feeding rates of a local nudibranch on invasive bryozoans, *Watersipora* and *Bugula neritina* (a complex with invasive species) and locally resident invertebrates (Craig's Lab). In addition, at SJSU (in collaboration with Okuda's Lab) we are comparing the toxicity of small-molecule fractions extracted in methanol from a range of fouling organisms, using brine shrimp as a repeatable assessment system, and are also using this same bank of chemical fractions to look for activity against sirtuin proteins, regulators of cancers and aging related diseases, through a genetic-reporter assay in yeast (Amagata's Lab).

In generating microsatellite loci for *Watersipora* species, we spent time with Dustin DeMeo and Michael Doan, and Robert Kam (at SJSU). These well qualified individuals helped with their coding and bioinformatics backgrounds. We established the use of a Linux server with four processers in the SJSU Conservation Genetics Lab, utilizing simple precedures to use remote computing resources, such as the NSF-support DIAG grid and the Amazon Cloud. As a result of various collaborative opportunities spawned by this project, we are developing papers on *Watersipora* genes and appoaches to identifying genes in non-model organisms.

This knowledge and ideas stemming from bioinformatics research has had an impact on teaching that is cross disciplinary. Students at SJSU have been exposed to gene-prediction based questions in two classes that have involved hands-on hypothesis testing, taught by Dr. Joshua Mackie: (1) Bio255E *Graduate Seminar in Eco-informatics*, fall 2013; and (2) *Bio145*, *Topics in Bioinformatics and Computational Biology* Seminar, spring, 2014. This development of computing expertise has lead to courses that have been cross-listed in two departments at SJSU: Biological Sciences and Engineering (Bioinformatics submajor).

We are in a vibrant area of research, and sometimes surprising linkages have developed from this experience. Misty Peterson, a Master's student at SJSU, is completing a research project in Dr Rohinton Kamakaka's yeast genetics laboratory at the University of Santa Cruz (examining committee: Drs Bros and Mackie at SJSU, along with Dr. Kamakaka at UCSC--prior to her entering the PhD program at the University of Kent). Misty's interest in copper regulating genes, fueled by the Bio255E class she took with Dr Mackie, lead to the realization that duplications of CUP-1 in yeast, which elevates the ability of yeast to survive copper-dosing when grown in the lab, can be used as a marker for measuring mutation rates. The experimental system that can be developed (and is the subject of Dr. Kamakaka's research) allows one to address such questions as: do mutations at certain loci predict genome instability, and the quickening pace of evolutionary adaptation of the genome overall?

What is the impact on the development of human resources?

This grant has enabled Dr. Sean Craig (HSU) to provide exposure to science and technology for the public, particularly young people (grades 4-8) learning science at the McKinleyville Middle School. Dr. Craig offered a GATE course (3 sessions of 5 hours each = 15 contact hours total) for Gifted And Talented Education students in 4-8th grades (25 students total). This group learned from Dr. Craig (and live specimens he brought in to McKinleyville Middle School) at their learning site as well as (for 1 day) at the Telonicher Marine Lab in Trinidad, California-where students gained a tour of this research project (the Telonicher Marine lab is a part of Humboldt State University, and is where *Watersipora* colonies were raised in common garden experiments by Dr. Craig and Kellan Korcheck).

In addition, with added funding provided by a collaboration with Dr. Jan Hodder (at the Oregon Institute of Marine Biology), Dr. Sean Craig co-taught a 1-week COSIA (Communicating Ocean Sciences to Informal Audiences) course (at the HSU Natural History Museum & Telonicher Marine Lab) w/Dr. Jeffrey White (May 21-25, 2012). This course trained graduate students, a few exceptional undergraduates, as well as some local professionals in effective outreach techniques to translate complex science concepts (including marine bio-invasions) and communicate them to the broader public.

This NSF funded project has also improved the performance, skills, and interests in Science of several underrepresented groups, improving their retention in research and teaching-related professions.

Our research provided multiple opportunities for undergraduate and graduate students at both institutions for training and professional development. Presentation of results at scientific meetings by most of our undergraduate and graduate students built further confidence in their ability to do science themselves. In addition, summer fellowship experience provided an opportunity for students to do hands-on science at a time when decreasing funding for the CSU is leading to fewer and fewer laboratory and field experiences for undergraduate students (see previous section for details).

Hands-on experience in different aspects of this project assisted in the progression/development of undergraduate students to the next stages of their careers involving Science at both institutions (San Jose State University & Humboldt State University). Several of our students (N= 3) went on to other paid scientific positions (eg., Martin, Ho, Chen) or to further study in Biology in graduate programs (N=4, including Reginald Blackwell, Danielle Perryman, Seena Sajadieh, and Parham Tabar). Notably, 3 of the students involved in this research project (N=12 total undergraduates) who went on to further graduate work were minority students, all of whom (100%) are now in graduate school pursuing Master's (n=2) or PhD degrees (n=1). Two of these students are African Americans, a group very poorly represented in marine science, who were recently accepted into graduate programs: (1) Danielle Perryman (SJSU), who was supported through the NSF-RISE program at SJSU, graduated and was accepted to Dr Jennifer Grindstaff's Laboratory at Oklahoma State University to conduct graduate research into endocrine systems and ecology in their Masters-PhD program, and (2) Reginald Blackwell (HSU), who was supported by an NSF-URM program as well as this NSF-RUI grant, graduated and was accepted into the PhD program to work in Dr. Ron Burton's Laboratory at Scripps Institute of Oceanography, contiuning study of marine molecular ecology. In fact, Regginald Blackwell landed a prestigious 3-year NSF pre-doctoral GFRP fellowship (approx $100,000) to pursue this degree!

All told, this project helped to spur on the completion of undergraduate degrees by eleven (11) of the twelve (12) undergraduate students involved (1 is still enrolled at UC Berkeley to complete his degree). In addition, of the 12 undergraduate students (total) involved in this NSF funded project, seven (7/12 = 58%) have gone on to graduate programs in the sciences.

This project has also lead to a great deal of productivity by our graduate students: A total of 14 graduate students have been supported, at least in part, by this NSF grant, and eleven (11) of these (11/14 = 78%) students completed master's theses related to this research project (see previous section for details).

Finally, this research grant helped the two co-PIs, Drs. Mackie and Craig, develop additional courses at SJSU and HSU, respectively, in the areas of Bioinformatics and Advanced Invertebrate Zoology at their respective institutions which will continue on into the future.

What is the impact on physical resources that form infrastructure?

The Genetic data analysis server which was developed at San Jose State University (SJSU) reflects a small step in addressing the generally well recognized need to build increased capacity for Bioinformatics infrastructure shared by the Departments of Biology and Engineering at SJSU. This funded project had an influence on a collaborative grant to increase infrastructure resources at SJSU, presented in an unsuccessful NSF-MRI proposal, (NSF Proposal: 1532051, *Acquisition of Hybrid CPU/GPU High-Performance Computing and Storage for Multidisciplinary Research and Teaching at San Jose State University* (NSF, MRI, submitted, Jan 22, 2015), PI: Sen Chiao - Meteorology, College of Sciences SJSU, co-PIs: Alison Bridger (Meterology), Guangliang Chen (Mathematics), Ehsan Khatami (Physics), Brooke Lustig (Chemistry), Joshua Mackie - Biological Sciences, Aaron Romanowsky – (Physics), Kamran Turkoglu (Aerospace Engineering)). The goal of this proposal and ongoing collaboration across departments is to acquire a state-of-the-art high-performance computing (HPC) facility to support a wide variety of research projects and teaching activities at San José State University (SJSU) as a key hub for STEM fields in the San Francisco Bay Area. Seeing a future impact, we believe the experience gained in this project will generally enhance our ability to bring enhanced technological resources and teaching to be used across CSU campuses.

What is the impact on institutional resources that form infrastructure?

The research pursued at San Jose State University (SJSU) with this funding was used to help provide additional BioInformatics program support, including the development of several courses by Dr. Joshua Mackie: (1) Bio255E *Graduate Seminar in Eco-informatics*, fall 2013; and (2) *Bio145*, *Topics in Bioinformatics and Computational Biology* Seminar, spring, 2014. This development of computing expertise has lead to courses that have now been cross-listed in two departments: Biological Sciences and Engineering (Bioinformatics submajor) at SJSU.

In addition, the research supported by this NSF grant lead to two courses at Humboldt State University taught by Dr. Sean Craig: (1) A seminar on *Bio-Invasions (Biol 685)*, offered to graduate students, as well as refinements in (2) an upper-division elective course for marine biology majors at HSU (as well as graduate students) entitled *Advanced Invertebrate Zoology (Zool 552)*.

What is the impact on information resources that form infrastructure?

The research pursued at San Jose State University (SJSU) with this funding was used to help provide additional BioInformatics program support, including the development of several courses by Dr. Joshua Mackie: (1) Bio255E *Graduate Seminar in Eco-informatics*, fall 2013; and (2) *Bio145*, *Topics in Bioinformatics and Computational Biology* Seminar, spring, 2014. This development of computing expertise has lead to courses that have now been cross-listed in two departments: Biological Sciences and Engineering (Bioinformatics submajor) at SJSU In addition, the Genetic data analysis server at SJSU was developed at San Jose State University as part of this research effort-and this server will help to hold data and results from further bioniformatics research efforts at SJSU, and enhance collaborations across fields. Very much as a result of this experience, we are more clearly recognizing the need to develop cross-departmental informational resources (as described above under physical resources), and useful inter-deparmental and inter-campus strategies for enhancing computing.

What is the impact on technology transfer?

Technologies or Techniques - Development of Public Use Tools

- **Development of a pipeline for predicting gene function, using *Watersipora subtorquata***

Raw DNA sequence for characterizing *Watersipora subtorquata* was provided by the Mackie Lab at SJSU. The genomic data consists of 42 million Illumina MiSeq paired-end reads (100 bp each end). The library was prepared with standard Illumina primers and Illumina TruSeq Adapter 12 indexes. The reads are compressed into two fastq files, one for each paired end, which are ordered sequentially. The data is processed through an Ab-initio gene prediction pipeline on a Dell R905 server with 16 2.2Ghz cores, 64Gb RAM, and 1TB of hard drive space.

The Ab-initio gene prediction pipeline includes assembling the raw reads and identifying putative genes within the assembled contigs (contiguous sequences) from homologues in GenBank. The pipeline includes options for trimming

ends of sequences, setting sequence accuracy parameters and for gene searches using the BLAST or HMMER algorithms. All commands and BioPython scripts are publicly available and under version control at: https://bitbucket.org/dustindemeo/abinitio/src. The scripts and results of an initial set of assemblies to retrieve sequences of genes in *Watersipora* will be described in a future publication.

- **Development of a distribution-free test of population-level aggregation of sequence haplotypes**

A number of research avenues require a test to determine whether clumping of like-objects in one observation set (or sample) is higher than would be expected by chance. In analyzing COI sequences of haplotypes under different scenarios - in larval data sets (for *Cancer magister* and *Neotrypaea californiensis*) and colonies of the bryozoan *Watersipora subtorquata*, we became aware of the need to develop a test to determine whether repeat occurrence of an exact 'haplotype' (a linked set of nucleotides, usually a region of a DNA strand) are found within a biological sample. High numbers of co-occurrences of the same mitochondrial haplotype may indicate cohort-dispersal of larvae from the same female parent (or maternal colony, as in the case of a hermaphroditic bryozoan), indicating that larvae found traveling together are from the same parental colony.

We developed a random-draw test that accepts population-delimited sequences as input to test the following:

If counts of co-occurrence in sampled subpopulations exceed the expected counts due to chance alone, then the observation is consistent with a higher rate of familial aggregation in the sample than would be expected within the general population.

Being able to recognize this numerical property allows more exact identification of biological dispersion phenomena in which contact between related individuals is maintained over time. The test, which is known as the Distribution Free Aggregation Test (DFAT), is useful for re-analyzing published haplotype data sets (Mackie and DeMeo in prep.). DFAT can be run currently with an installation of BioPython on PC or Mac. All commands and BioPython scripts are publicly available and under version control at: https://bitbucket.org/dustindemeo/dfat/src

What is the impact on society beyond science and technology?

The results of this research are likely to make an impace on the practices used to prevent hull-fouling on ships and boats. Because our results show that multiple species, and not just one species of Watersipora, have now evolved a tolerance to copper in anti-fouling paints used on ship hulls, it seems likely that further development of alternative anti-fouling paints or coatings will be necessary. In fact-the research in this NSF-funded project suggests that these anti-fouling paints may be, in effect, assisting certain species in invading around the world!

Changes/Problems

Changes in approach and reason for change

In July, 2013 at the request of the Humboldt State Foundation, Dr Leslee Parr was made a surrogate PI to stand-in for Dr J. Mackie. This was to ensure financial duties could be handled through the San Jose State University Research Foundation during a period in which Dr. Joshua Mackie, an Australian, transitioned in visa status from a J1 Visa sponsored by San Jose State University, to hold a US Green Card. This visa transition was completed (September 2014) and Dr. J. Mackie was re-established to co-PI status.

Actual or Anticipated problems or delays and actions or plans to resolve them

Some delays in the preparation of publications resulting from this research have occurred at Humboldt State University due to a serious illness in the family of Dr. Sean Craig: his daughter (age 16) has been diagnosed with Ehlers-Danlos syndrome, a genetically inherited disease affecting connective tissue, and specficially the collagen proteins found in the human body. This illness has required Dr. Craig to spend a lot of extra time caring for his daughter (and teaching her at home-as she is unable to attend public school) during the final 2 years of this project.

Changes that have a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use or care of biohazards
Nothing to report.